Pathology of the Glomerulus in Sickle Cell Anemia With and Without Nephrotic Syndrome

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Glomeruli from 6 cases of sickle cell disease (SS) with the nephrotic syndrome (NS) were compared histologically and quantitatively with glomeruli from 9 cases of SS, 10 cases of sickle cell trait (SCT), 4 cases of other hemoglobinopathies, all without NS, and normal controls. Five of 6 patients with SS and NS had extensive reduplication of their glomerular basement membranes and mild mesangial proliferation. Similar but milder lesions occurred in SS without NS but not in SCT or controls. Incidental renal disease occurred in 1 patient with SS and NS. Nephrotic syndrome was probably secondary to effects of sickle cell disease. Glomeruli in SS were significantly larger (>70%) than in SCT and controls. Mean total glomerular area per unit area of cortex in SS with normal BUN significantly exceeded that of SCT, which, in turn, was significantly greater than that of controls. Mechanisms for the histologic lesions and hypertrophy of the glomeruli were suggested (Am J Pathol 77:357–376, 1974).

IN THE PAST, little attention has been paid to the pathologic changes in the kidney in sickle cell anemia. Recently there have been a number of reports of the association of sickle cell anemia and the nephrotic syndrome.[1–11] The reported renal cortical changes in sickle cell anemia without nephrotic syndrome[6,12–14] include: small cortical infarcts (of varying ages),[12] hemosiderin deposits in the epithelium of the proximal convoluted tubules, dilatation of afferent and efferent arterioles, glomerular congestion, hypertrophy of juxtamedullary glomeruli[13] and eventual glomerular sclerosis. Recently, Pitcock *et al*[15] and Arakawa and Kimmelstiel[16] reported focal reduplication of the glomerular basement membranes and some mesangial proliferation. The patients with both sickle cell disease and the nephrotic syndrome have had reduplication of the glomerular basement membranes,[1,5] some mesangial proliferation,[5] iron-containing deposits in glomerular epithelial cells,[5] and glomerular sclerosis. Controversy exists over whether or not there is a causal relationship between sickle cell disease and the nephrotic syndrome and, if there is, what is its pathogenesis.

To better understand the renal cortical changes in patients with sickle cell disease and the nephrotic syndrome, we reviewed the clinical

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records and autopsy tissues of patients with sickle cell anemia (SS), sickle cell trait (SCT), and other hemoglobinopathies. This paper reports the pathologic changes in the renal cortex in 6 patients with SS and the nephrotic syndrome (NS), 2 with SCT and NS, 9 with SS without NS, 10 patients with SCT, and 1 patient each with S-thalassemia, S-C disease, C-C disease and S-F heterozygous trait. Quantitative studies of glomerular size in SS are compared with SCT and normals. Possible etiologies of the glomerular lesions and hypertrophy of the glomeruli are discussed.

Materials and Methods

Renal tissue was obtained by percutaneous needle biopsy from 8 patients with the nephrotic syndrome and SS or SCT and from autopsies in the other patients save for one partial nephrectomy for unilateral hematuria. Since blocks of tissue were obtained from several institutions, all blocks were recut at 3 μ and stained with hematoxylin and eosin, PAS, periodic acid–methenamine silver (PAM) and the Prussian blue reaction. Tissue for electron microscopy and fluorescent microscopy was available from 4 and 3 patients, respectively, with SS and NS, and was prepared as previously described.[17]

Antisera, obtained from Hyland Laboratories, were labeled with fluorescein isothiocyanate (FITC) in a ratio of 0.02 mg FITC/mg protein, separated from unbound FITC on Sephadex G-25 columns, and adsorbed with beef liver powder. Each unlabeled antiserum gave a single line when immunoelectrophoresed against pooled normal human serum. The following antihuman antisera were used: anti-IgGAM, anti-IgG, anti-IgA, anti-IgM, anti-complement C3, anti-IgE, anti-IgD, antialbumin, and anticeruloplasmin. No deposits occurred in normal kidneys obtained at nephrectomy for tumor or at autopsy. Appropriately positive results were found in over 100 cases of lupus, membranoproliferative glomerulonephritis, idiopathic membranous glomerulonephritis, Henoch-Schonlein purpura and other diseases. No glomerular deposits of antialbumin or anticeruloplasmin have been detected.

Glomerular sizes were determined by ocular micrometry. The two longest axes at right angles to each other were measured. Surface area, or "size" was calculated by the formula $A = \pi/4XY$. The validity of measurements by ocular micrometry was checked by photographing a selected group of previously measured glomeruli, projecting the photographs, comparison with the projection of a photograph of a stage micrometer at the same magnification, and comparison of areas obtained by the cut-out and weigh method that has previously been described.[18] In all cases, 30 of the larger glomeruli were measured by ocular micrometry. Since glomeruli are essentially randomly cut at any angle or level in any single section, measuring the larger glomeruli should give a closer evaluation of maximal glomerular size. This was confirmed by serially sectioning 2 cases, staining every other section, measuring all glomeruli in the middle section and following each glomerulus to its maximal size and measuring. In 1 case the mean size of all glomeruli in one section was 39,100 sq μ, while the mean size of the same glomeruli at largest diameter was 53,200 sq μ. When the larger glomeruli only were selected from the same case, the mean size was 51,600 sq μ.

The number of glomeruli per unit area, hereafter referred to as the density, was determined by counting all glomeruli, including sclerotic ones seen in a 36-square ocular micrometer at 12.5 × 2.5 magnification. At least three and gen-

erally six to eight fields were counted, and the mean density was determined. Controls for both glomerular size and density were studied from normal autopsied kidneys obtained from the medical examiner's office.

Clinical records of all patients were reviewed. Glomerular sizes were correlated with age, mean blood pressure, hemoglobin level, heart weight, kidney weight, density of glomeruli and presence or absence of congestive heart failure. Data were not available to correlate with total body surface area. More detailed clinical data are presented elsewhere.[19]

Results

Clinical Observations

Tables 1 and 2 summarize the clinical data on all patients, organ weights and causes of death in the autopsied patients. The urine specific gravity and amount of proteinuria were performed on either admission or random samples of urines. No patients had urinary concentration tests and only the patients with nephrotic syndrome had 24-hour urine protein and creatinine clearances.

The first group (Table 1) consists of the 6 patients with SS and NS. The autopsied patients are arranged into two groups (Table 2) according to the type of hemoglobinopathy: SS and SCT. The histories and results of clinical and morphologic studies in 2 patients (8830 and 73-2292) were consistent with a diagnosis of glomerulonephritis. Patient 8830 had a chronic proliferative glomerulonephritis with progressive renal failure. Patient 73-2292 had a history of streptococcal infection, followed in 2 weeks by severe oliguria, and a rise in antistreptolysin titers; after diuresis he became nephrotic. Eight patients had congestive heart failure. Alcoholic cardiomyopathy was thought to be the etiology in 3 patients. Arteriosclerotic heart disease was the etiology of the heart failure in 4, while sickle cell disease with hypertension and chronic renal disease were the presumed cause in 1. Significant elevation of either the BUN or serum creatinine was found in 7 patients. In 3 patients this was due to acute renal failure and in 2 to glomerulonephritis; in 2 patients severe intrarenal arteriosclerosis and the changes of sickle cell disease caused chronic renal failure. Established hypertension was present in 2 patients with SS. None of the autopsied patients had confirmed nephrotic syndrome, although random urine samples showed 3+ proteinuria in two, 2+ proteinuria in one, 1+ to 3+ proteinuria in one, and 1+ to 2+ proteinuria in one.

Morphologic Observations

Table 3 summarizes the morphologic changes. Iron deposits were graded on a scale of 0 to 2+. A grade of 1+ in glomeruli meant a few

Table 1—Clinical Data on Six Patients with Sickle Cell Disease with Nephrotic Syndrome (NS) (Group I)

Case/sex	Age (yr) (onset–present)	Hgb (g%)	CHF	Duration (yr)	BUN (mg%)	Creatinine clearance (ml/min)	BP	24-hr urine protein (g)	Urine (sp g)	Albumin (g%)	Cholesterol (mg%)	Other
68-25/F	10–14	7.3	no	—	36	36	80/60	14.2	1.012	1.8	490	
71-5565/F	12–15	9.1–9.7	no	—	12	107	118/68	7.7	1.012	1.8	490	
71-280/F	23–25	5.6–8.2	no	—	6	121	110/70	8	1.008	1.3	298	
70-5155/F	24–27	6.9–8.4	no	—	20	30–50	130/80	19	1.007	1.6	390	
71-408/M	31–33	6–7	yes	2–3	48	60	160/100	13.4	1.012	3.9	222	Died
73-2292/M	11–11	6.0	no	—	125 →6	15–76	125/75	5.1	1.011–1.012	1.8	230	Strep. throat followed by AGN then NS

CHF = congestive heart failure, AGN = acute glomerulonephritis

scattered epithelial cells contained iron. A grade of 2+ in glomeruli indicated that over 20% of cells contained iron. The column labeled arteriosclerosis was an evaluation of the arcuate and intralobular arteries. Two patients had interstitial edema, tubular dilatation with flattened epithelium and other changes of acute renal failure which has been designated tubulointerstitial nephropathy (TIN). Three patients had significantly abnormal small medullary vessels. In 2 the vasa rectae appeared to have become aneurysmally dilated, and in the third the vasa rectae looked like small glomeruli having many small basement-membrane-lined channels. In all 3 these lesions of the vasa rectae were associated with loss of medullary tubules and medullary fibrosis.

Pathologic Changes in Sickle Cell Anemia with Nephrotic Syndrome (Group I)

Five of the 6 patients with SS and NS had essentially identical histologic changes. They had focal or diffuse reduplication of the glomerular basement membranes (GBMs) (Figure 1) and segmental or diffuse sclerosis of the glomeruli. The completely sclerosed glomeruli could not be distinguished from ischemic sclerosis. Several glomeruli had obvious ischemic wrinkling and thickening of the GBMs. Mesangial proliferation was mild to moderate and tended to be focal. Other changes included adhesions and small crescents in 4 of 5 patients. The sixth patient (73-2292) had documented poststreptococcal proliferative glomerulonephritis (AGN). His biopsy had a few capillary loops with reduplicated GBMs. Stainable iron was present in a few glomerular epithelial cells in 2 of 6 cases and in proximal convoluted tubules of all 6 cases. Sickled red cells distended the capillary loops of all 6 cases.

Four patients, including the one with AGN, had electron microscopic studies. Three patients had essentially the same changes. Reduplication of the GBMs was significantly more extensive than was appreciated by light microscopy. Mesangial cell cytoplasm was interposed between the outer original GBM and the inner basement-membrane-like material (Figures 2 and 3). No electron-dense deposits were observed. The reduplications of the GBM involved the entire periphery of some capillary loops and only segments of others. The minority of capillary loops were either unaffected or had irregular thickenings with loose material in the subendothelial region. In the reduplicated GBMs, the outer membranes were often thicker than usual. Nonreduplicated GBMs ranged from 2200 to 5500 Å in thickness, and in reduplicated areas they measured up to 11,000 Å in thickness. Scattered unbound aggregates of electron-dense granules, approximately 250 Å in diameter, and an occasional small membrane-limited siderosome were seen in endothelial and

Table 2—Clinical Data on Autopsied Patients, Nine with Sickle Cell Disease (Group II)

Case/sex	Age (yr)	Hgb (g%)	CHF	Duration (yr)	BUN (mg%)	Creatinine (mg%)	BP
Group II							
71910/M	10/12	8.4–4.8	no	—	14	—	140
A63-76/F	2	5–6	no	—	—	—	90/60
A63-57/F	7	5.0	no	—	—	—	—
8830/M	11	7.0	no	—	222	—	105/80
72414/M	22	7.8–8.6	no	—	13	1.2	150/80
A72-276/F	27	8	no	—	11	0.8	95/50
71867/F	42	6.1	no	—	16	—	95/60
A72-1/M	56	8.0–6.1	yes	3½	80–120	6.1	160/90
71593/M	59	4.6	no	—	77	—	130/80
Group III							
A60-98/M	7/12	—	no	—	—	—	—
A61-88/F	5½	5.1–5.5	no	—	—	—	150/70
75741/M	20	16.5	no	—	11	—	140/80
75423/M	27	14.2	yes	⅓	—	1.4	Narrow pulse pressure
A69-261/M	36	13.8–11.1	yes	⅓	12	1.0	120/90
72-5818/F	39	8.3	no	—	10	—	110/70
74560/F	41	14.6	no	—	10	—	140/100
76179/F	41	10.4–12.2	no	—	12–61	1.5–2.2	150/85
75643/F	52	12.2	yes	1+	—	2.4	130/90
75607/M	55	11	no	—	17	—	140/82

mesangial cells. The siderosomes in the glomeruli were smaller and less granular than those in the tubular epithelium. The patient with AGN (73-2292) had many characteristic subepithelial electron-dense deposits or "humps."

Fluorescent microscopy was negative on 2 patients with reduplicated GBMs. The patient with AGN had coarse granular deposits of both IgG and complement C3 on the GBMs with focal capillary loop deposits of IgM.

Two patients with sickle cell trait and the nephrotic syndrome (not included in the tables) had only minimal mesangial prominence in the

and Ten with Sickle Cell Trait (Group III)

Proteinuria	Urine (Sp g)	Kidney weight (g)	Heart weight (g)	Body weight (kg)	Body size (cm)	Other data
Trace	1.017	111	60	8.9	—	Sepsis
—	—	108	84	10	87	**E coli** sepsis, 50% reticulocytes
None	1.011	210 (nl 140)	125 (nl 100)	—	123	Liver failure and coma, spleen 295 g (nl 66)
4+	—	170	250	26	147	CGN, uremia, pneumonia, spleen 350 g
3+	1.012	—	—	—	—	Spleen 5 × 1 × .8 cm, bone infarcts, active TB
None	1.008–1.012	260	420	Thin	—	Thrombophlebitis, massive pulmonary embolization
None	1.009	400	280	Well developed	—	Pigment cirrhosis and liver failure
1+–3+	1.007–1.010	400	500	Poorly nourished	—	No spleen found, emphysema, chronic osteomyelitis
1+	1.006	355	420	68	164	
—	—	82	52	7	62	Pneumococcal sepsis, adrenal hemorrhage
—	—	98	148	14	112	Lead poisoning, spleen 16 g
Trace	1.030	335	350	—	—	Spleen 80 g, head trauma, alcoholism, diabetes
None	1.012	355	575	54	165	Alcoholic, alcoholic cardiomyopathy?
None	1.010	350	550	—	—	Alcoholic, alcoholic cardiomyopathy, Spleen 150 g, pulmonary embolism
None	1.019	NA	NA	50	—	Gross hematuria, partial nephrectomy
2+	1.010	400	300	WD	WN	Coma, diabetes, chronic pancreatitis, spleen 50 g
None	1.010–1.012	350	375	—	—	Diffuse organizing pneumonia, spleen 125 g
1+	1.007	275	325	Obese	—	No gall stones
1+	1.007	300	375	—	—	DTs, suicide, spleen 500 g

biopsy specimens. The histologic findings were inconsistent with lipoid nephrosis or nil disease, and they responded dramatically to steroids.

Light Microscopy in Sickle Cell Disease (Group II)

Of the 9 patients with SS, 3 children had normal appearing GBMs in nonsclerotic glomeruli, while the child (8830) with chronic glomerulonephritis had mesangial proliferation and irregularly thickened GBMs. One of 5 adults had rare reduplication of GBMs, 2 had many thick GBMs and in 1 there probably was GBM reduplication; 2 patients had definite reduplication. This varied from a few reduplicated GBMs

Table 3—Morphologic Data on Six Patients with Sickle Cell Disease with Nephrotic Ten Patients with Sickle Cell Trait (Group III)

Case	Glomeruli		Mesangial changes	GBM reduplication	Ischemic glomeruli	Iron	
	Area (sq μ)	Density				Tubules	Glomeruli
Group I							
68-25	15,600 ±5300	—	mild dif all, few foc	Most glom, most GBM	None	++	+
71-5565	10,800 ±3300	—	Few glom foc	Few glom, focal	Few	+	0
71-280	20,400 ±6300	—	Mild dif all, mod foc few	Most glom, most GBM	Few	+	0
70-5155	28,000 ±17600	—	All more foc than dif	All glom, many GBM	Up to half	+	0
71-408	43,400 ±14600	—	Mild dif all, some mod foc	All glom, most GBM	Few	++	0
73-2292	31,000 ±8000	—	Sev prolif exudate	Few glom, few BGM	None	++	+
Group II							
71910	8200 ±1000	>150	None	None	Mod No. subcapsular	0	0
A63-76	13000 ±1400	92.5	None	None	Few subcapsular	++	+
A63-57	13800 ±2600	44.8	None	None	Rare	0	0
8830	24200 ±4000	25.6	Sev prolif	None, thick GBM	Few	+	+
72414	37000 ±6100	50.8	Rare	Many thick probable dupl	Rare	+	+
A72-276	51600 ±11700	40.9	None	Rare	Few segmental, sclerosis	++	+
71867	42000 ±7700	38.5	Min in few glom	Most glom, GBM	Foc in scar, scattered	++	+
A72-1	41400 ±12200	27.2	Few glom foc	Some glom, few BGM	Mod No.	++	+
71593	39800 ±10400	19	Mod dif & foc prolif	Most glom, many GBM	>$\frac{1}{3}$ sclerotic, 8 of 59 ischemic	++	+
Group III							
A60-98	6400 ±700	>150	None	None	Mod No. subcapsular	0	0
A61-88	11800 ±2700	98.3	None	None	Few	++	+
75741	24800 ±4500	66.3	None	None	Rare	0	0
75423	22800 ±3900	46	None	None	Few	0	0
A69-261	26000 ±3600	43.7	None	None	Few	0	0
72-5818	25400 ±5700	41.9	None	None	None	0	0
74560	21600 ±4200	44.5	None	None	None	0	0
76179	23000 ±4600	57.8	None	None	Rare	0	0
75643	30200 ±5300	58.3	None	None	Few	0	0
75607	23800 ±4900	43.5	Rare	None	Few	0	0

Dif = diffuse, glom = glomeruli, foc = focal (ly), prolif = proliferation, sev = severe, min = minimal, mod = moderate, GBM = glomerular basement membrane, lin = linear

Syndrome (SS and NS) (Group I), Nine Patients with Sickle Cell Disease (Group II), and

Medullary scars	Infarcts: Fresh	Infarcts: Old	Arterio-sclerosis	Tubular atrophy	Fibrosis	Other
—	0	Yes 2nd	0	0	0	Few adhesions and crescents
—	0	0	0	Mod	Foc	Some segmental sclerosis
—	0	0	Min	Foc	Foc	Few adhesions, some segmented sclerosis
—	0	0	Min	Foc	Foc	Few adhesions and crescents
—	0	0	Min, mod	Foc	Min, dif	Few adhesions, fibrous crescents
—	0	0	0	0	0	Acute glomerulonephritis vasculitis
None	0	0	0	0	0	
None	0	0	0	0	0	
None	0	0	0	0	0	Tubulointerstitial nephopathy
—	0	0	Min	Sev	Sev	Chronic glomerulonephritis
None	Few	0	0	0	0	
None	0	2 lin	0	Foc	Foc	
—	0	2 lin	Min	Foc	Foc	
Atrophic sev tubular loss	0	0	Mod, sev	Sev	Sev	
Dif tubular loss	0	lin	Sev	Mod	Mod	Aneurysms of vasa rectae
None	0	0	0	0	0	
None	0	0	0	Rare	0	Lead poisoning, nephrocalcinosis
Min tubular loss	0	0	Min	0	0	
Min tubular loss	0	0	Min, mod	Foc	0	
Small old linear	0	Yes	Min, mod	Foc	0	
Foc tubular loss	0	0	Min	0	0	
Focal	0	0	Mod	Min	0	"Glomeruloids" of vasa rectae
None	0	0	Min	0	0	
Min tubular loss	Yes	Yes	Min	0	0	
Two small old	0	0	0	Foc	0	

in some glomeruli to many reduplicated GBMs in most glomeruli (71593). There was no correlation between the amount of proteinuria and GBM reduplication. Mesangial proliferative changes were severe in the patient with chronic glomerulonephritis; in the other 8 patients of group II, mesangial proliferative changes were moderate diffuse and focal in 1, mild and focal in 2, rare in 1 and absent in 4. Both GBM reduplication and mesangial proliferation increased in frequency with increasing age. Glomerular ischemia and sclerosis were more frequent in sickle cell disease than in sickle cell trait. Some glomeruli had segmental sclerosis. The slight glomerular deposits of iron were confined to glomerular epithelial cells.

Light Microscopy in Sickle Cell Trait (Group III)

The 10 patients with SCT had virtually normal appearing glomeruli. Only 1 patient had mild mesangial proliferation in rare glomeruli. All GBMs appeared to be delicate. Only 1 of 10 with SCT had any iron deposits. Extensive tubular and mild glomerular iron deposits were present in the patient with lead poisoning (A61-88), as were deposits of calcium salts (calcium versenate had been given).

Light Microscopy in Other Hemoglobinopathies

The histologic changes in this group were heterogeneous. Only the changes in the patient with S-thalassemia were notable. His kidneys were undistinguishable from kidneys in SS. A few glomeruli had slight mesangial proliferation and a few had reduplication of a few GBMs. Small amounts of iron were present in tubules but not in glomeruli. Medullary scarring with aneurysmal dilatation of the vasa rectae was also present.

Quantitative Studies of Size and Density of Glomeruli

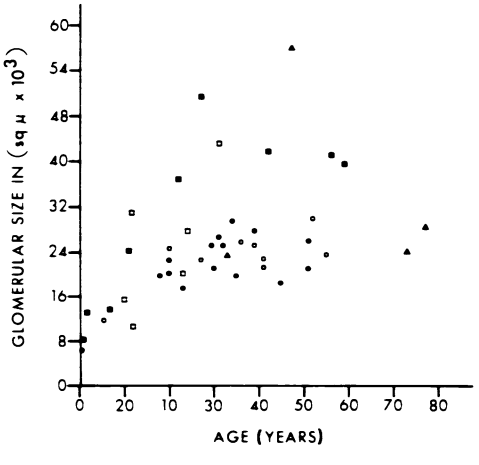
Table 4 summarizes the data of mean glomerular size and density in adults with SS with and without nephrotic syndrome, in adults with SCT, and in control patients. The children with SS and SCT were eliminated from analysis because there were too few to be statistically significant. The 15 controls ranged in age from 18 to 51 years. The mean size with standard deviation of their glomeruli was 23,000 $\pm$ 3600 sq μ with a range of the means from 17,600 to 29,600 sq μ. The glomeruli of the 8 adults with SCT, whose mean size was 24,700 $\pm$ 2,500 sq μ with a range of 21,600 to 30,200 sq μ, did not statistically differ in size from the controls by the student t test: $t = 1.64$ and $P > 0.10$. The mean glomerular size of the five adults with SS was 43,000 $\pm$ 6,200 sq μ with

Table 4—Comparison of Glomerular Sizes, Densities and Total Areas in Adults

Group	No. of cases	Mean glomerular areas (sq μ) ± SD	Mean density of glomeruli/unit area of cortex	Total glomerular area/unit area of cortex (sq μ)
Controls	15	23,000 ±3,600	46.4 ± 8.2	10,550 × 10^2 ±1,790
Sickle cell trait	8	24,700 ±2,500	48.4 ± 10.1	12,480 × 10^2 ±3,020
Sickle cell disease	5	43,000 ±6,200	35.3 ± 11.1	14,980 × 10^2 ±5,530
SS disease witn NS	3	33,200 ±10,500	—	—

a range of 37,000 to 52,000 sq μ. The glomeruli of patients with SS were 87 and 74% larger, respectively, than controls and SCT. The difference in glomerular sizes was significant: for the controls $t = 7.617$ and $P < 0.001$ where $f = 18$; and for SCT, $t = 8.090$ and $P < 0.001$ where $f = 11$. To make the data from the 3 adults with SS and NS comparable with the other groups, the small sclerotic and tangentially cut glomeruli were eliminated. The newly derived mean size was 33,200 ± 10,500 sq μ. The glomeruli from the SS and NS group were significantly larger than those of the controls ($t = 3.2017$ and $P < 0.005$ with $f = 16$) and SCT ($t = 2.470$ and $P < 0.05$ with $f = 10$).

Mean glomerular sizes were plotted against age in Text-figure 1. Glomerular size varied little with age in the controls, although there probably were not enough controls to accurately determine differences in size by decade. While glomerular size in the SS group increased with age, it was not strictly age dependent. The increased cross-sectional



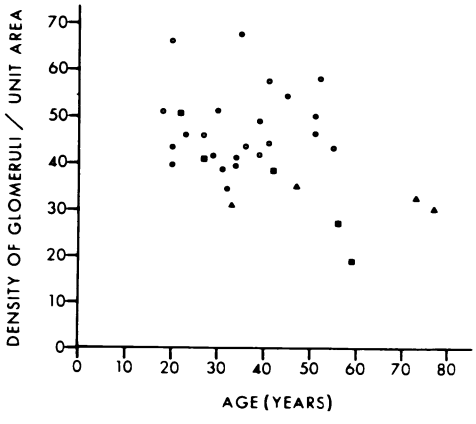
TEXT-FIG 1—Glomerular size vs age in hemoglobinopathies and controls. (SS hemoglobin, *solid squares*; SS + nephrosis, *open squares*; AS + nephrosis, ±; AS hemoglobin, *open circles*; other hemoglobins, *solid triangles*; controls, *solid circles*).

area of glomeruli of the SS group of 74 and 87%, respectively, over SCT and controls implies at least a 2.5-fold greater glomerular volume. The total available capillary surface area for glomerular filtration is probably much greater.

There was no correlation between glomerular size and various clinical and pathologic parameters, including mean blood pressure, BUN, presence of heart failure, heart weight or kidney weight. Hemoglobin concentration was inversely related to glomerular size, with two exceptions. The exceptions were: a 33-year-old male with CC hemoglobinopathy and a mean hemoglobin concentration of 5 g% had normal sized glomeruli (23,600 ± 4600 sq μ); and a 39-year-old female (72-5818) with SCT, admitted for massive hematuria, had a hemoglobin concentration of 8.3 g% and normal sized glomeruli (25,400 ± 5700 sq μ). The patient with S-thalassemia had the severe form with many crises and a clinical course identical to sickle cell disease. His glomeruli were the largest observed: 58,200 ± 8900 sq μ.

Glomerular densities in adults of all groups, except for the biopsied patients, were plotted against age in Text-figure 2, and the means for the groups are summarized in Table 4. There was no significant difference in mean density of glomeruli per unit area of cortex between controls and SCT. The differences in mean density between SS and either controls or SCT were significantly affected by the extensive intrarenal arteriosclerosis and consequent disappearance of obsolescent glomeruli in 2 patients (A72-1 and 71593). If those 2 patients are eliminated, the reduction in mean glomerular density in the SS group is 11 and 14% instead of 24 and 27%, respectively, when compared to controls and SCT.

The total glomerular area per unit area of cortex (TGAC) is ap-



TEXT-FIG 2—Glomerular density vs age in hemoglobinopathies and controls (SS hemoglobin, *solid squares*; AS hemoglobin, *open circles*; other hemoglobins, *solid triangles*; controls, *solid circles*).

proximated by the product of the mean glomerular area and the mean density. In SCT the mean TGAC was 18.3% greater than in the controls. This difference was significant: $t = 2.323$ and $P < 0.025$ (1 tail) with $f = 21$. The SS group had 20 and 42% greater mean TGAC than did SCT and controls, respectively. The difference in TGAC was not significant between SS and SCT but was significant between SS and controls: $t = 2.798$ and $P < 0.005$ with $f = 18$. When the 3 patients with SS and normal BUN were compared with the controls and SCT, the mean TGAC of the SS group ($18{,}690 \times 10^2$ sq μ) was 50 and 77% greater than SCT and controls, respectively. The differences were significant: for SCT, $t = 2.112$, $P < 0.05$ (1 tail) with $f = 9$; and for controls, $t = 4.194$, $P < 0.005$ (1 tail) with $f = 16$.

Discussion

In the literature, four different mechanisms have been proposed to account for the etiology of NS in patients with SS. Heptinstall [14] thought the association was probably fortuitious. This is most likely the case for patients with SCT and NS. Our 2 patients with SCT had biopsy-proven "nil" disease or lipoid nephrosis and responded appropriately to steroid therapy. Incidental lipoid nephrosis,[2] poststreptococcal glomerulonephritis [20] or other renal diseases have been reported in a few patients with SS as occurred in 2 of our patients (73-2292 and 8830). However, fortuitous renal disease does not account for the great majority of patients with SS and NS.

McCoy [5] thought that the nephrotic syndrome was due to iron overload in the glomerulus. He suggested that the mechanism was analogous to Ellis's [21] experiments where intravenously administered saccharated iron oxide caused NS in rabbits. In Ellis's experiments, large intravenous doses of saccharated iron oxide precipitated in glomerular capillaries, massively occluded them, and eventually led to a proliferative and fibrotic response. Ellis's early lesions [22] showed loss of foot processes of epithelial cells and many iron-positive granules but not reduplication of the GBMs. In the experiments of Dachs and Churg,[23] after multiple large intravenous doses of saccharated iron oxide more extensive lesions, which included large amounts of iron, were seen, especially in the mesangium. The mechanism of iron overload is unlikely in man in most cases because of the paucity of Prussian-blue-stainable iron in the glomeruli. Four of the 6 patients with SS and NS had no stainable iron in their glomeruli, while the other 2 had a few small scattered deposits, mainly in visceral epithelial cells. Patients with SS (group II) also had essentially only small amounts of iron in epithelial

cells, as did the patients reported by Pitcock *et al.*[15] Electron microscopy showed only a few discrete siderosomes and scattered aggregates of electron-dense granules in the glomeruli.

Pardo *et al*[7] demonstrated granular deposits of IgG on the GBM of at least one patient with SS disease and proteinuria. They suggested that because SS patients had more infections, they were highly likely to develop immune complex disease secondary to these infections, and thence the nephrotic syndrome. While this mechanism may possibly account for a small number of patients with NS, it probably does not explain most cases. Two of 3 of our patients had negative immunofluorescent studies. The 1 case positive by both fluorescent and electron microscopy had a proven streptococcal infection that preceded his acute glomerulonephritis. Neither Antonovych's 5 cases nor 3 of our cases had significant electron-dense deposits on the GBM that may have been suggestive of immune complexes.

Antonovych [1] suggested that "intracapillary fragmentation and phagocytosis of sickled red cells may play a role in the pathogenesis of the nephrotic syndrome in sickle cell anemia." All 5 of her patients had focal thickening and reduplication of GBMs with mesangial interposition between the membranes. Pitcock *et al*[15] indirectly contributed to this thesis by showing that the early renal changes in sickle cell anemia without significant proteinuria included mild mesangial proliferation and focal reduplication of GBMs. The same lesions were seen in several of our adults with SS only. Those with SS and NS had similar but more severe lesions. We speculate that the difference between those with NS and those without NS is not qualitative but rather of degree of severity of the same lesions.

We would further speculate that when the fragmented red cell masses are small they become impacted in isolated capillary loops and are phagocytosed by mesangial cells which simultaneously lay down new basement membrane material internal to the original GBM, resulting in the reduplicated appearance. Vassalli *et al*[24] have shown that various means of inducing intravascular fibrin formation cause similar lesions to develop in rats. Vitsky *et al*[25] have seen virtually identical lesions in patients with the hemolytic uremic syndrome who have been biopsied during the healing phase. Larger masses of fragmented red cells may occlude part or all of the capillary loops and thereby cause the frequently observed segmental sclerosis by either local ischemia, infarction or both.

Hypertrophy of the glomeruli in SS has been conclusively demonstrated by the quantitative studies which are summarized in Table 4.

Bernstein and Whitten [13] had noted that the juxtamedullary glomeruli were enlarged in children with sickle cell disease. In adults we found glomerular hypertrophy of approximately the same degree at all levels of the cortex. There was no relationship between glomerular hypertrophy and kidney weights, heart weight, presence of heart failure or mean blood pressure. There was an inverse relationship between glomerular size and hemoglobin concentration in SS, the patient with S-thalassemia and 2 of 3 patients with SS and NS. The mean total glomerular area per unit area of cortex was greater in SS than in either controls or SCT. This difference was further accentuated when only the SS patients with normal BUN were compared to controls and SCT who had normal renal function. Since the TGAC in sickle cell disease is so greatly increased, the glomerular hypertrophy must be more than just compensatory hypertrophy to replace the lost glomeruli.

Comparison with other diseases and conditions (Table 5) associated with glomerular hypertrophy may shed some light on its pathogenesis in SS. The following text table both lists the other conditions associated with glomerular hypertrophy and organizes them into a conceptual framework. The various nephritides and infiltrative diseases (diabetes and amyloid) are not included.

Hypertrophy of glomeruli in SS is clearly not related to conditions with loss of kidney parenchyma. It does share in common with the first group increased blood viscosity as well as tissue anoxia. The tissue anoxia in SS results from a reduction in the total concentration of hemoglobin rather than deficient oxygenation of the hemoglobin as in other groups. Tissue anoxia probably does not contribute significantly

Table 5—Comparison with other Diseases Associated with Glomerular Hypertrophy

Diseases with altered hemoglobin concentration and altered blood viscosity.
Increased hemoglobin concentration and increased blood viscosity.
Cyanotic congenital heart disease[26]
High altitude children[27] and rats[28]
Cor pulmonale[29]
Abnormal hemoglobins with low concentration and increased viscosity.
Sickle cell disease and variants.
Diseases with loss of kidney parenchyma
Single kidneys
Congenital
Iatrogenic
Bilateral hypoplasia with oligomeganephronia[18,30]
End stage kidneys with hypertrophy of the few remaining intact glomeruli.
Unclassified
Congestive heart failure[31]

to glomerular hypertrophy, as demonstrated by observations of our patient with CC hemoglobin who had a 4.5 to 5.6 g% hemoglobin concentration and normal sized glomeruli, and by Bernstein and Whitten's [13] observation of mild or no hypertrophy of juxtamedullary glomeruli in children with a variety of other severe chronic anemias. Hutchins and Kutchemeshgi [31] thought that the elevated venous pressure in congestive heart failure might lead to dilatation of afferent arterioles in order to decrease frictional resistance and maintain renal blood flow. They thought the resultant exposure of the glomeruli to blood under high pressures might be the stimulus for glomerular hypertrophy. This may be the final mechanism in heart failure, cyanotic congenital heart disease and cor pulmonale. In sickle cell disease the increased blood viscosity is caused by the abnormal hemoglobin when it is deoxygenated [32] and sickling has occurred. It could then cause increased frictional resistance. It has been noted by Bernstein and Whitten [13] and others that the afferent and efferent arterioles at autopsy are dilated and congested with sickled red cells.

Children [33] and young adults [34] with SS generally have supernormal glomerular filtration rates and renal blood flows. After the third decade these parameters decline.[35] There may be a relationship, as yet unexplained, between the supernormal renal function and the glomerular hypertrophy and the total glomerular area per unit area of cortex.

Because of the reduplication of the GBMs and the mesangial cell proliferation, the lesions in SS and NS have been confused with hypocomplementemic membranoproliferative glomerulonephritis (MPGN). The two entities can be distinguished on both clinical and pathologic bases. In MPGN the complement C3 is low in serum. Granular deposits of complement C3 are characteristically deposited in a peripheral lobular distribution on the GBMs. The glomeruli are lobulated due to marked mesangial cell proliferation, and the peripheral capillary lumens are considerably narrowed. None of these clinical or pathologic features are present in patients with SS and NS.

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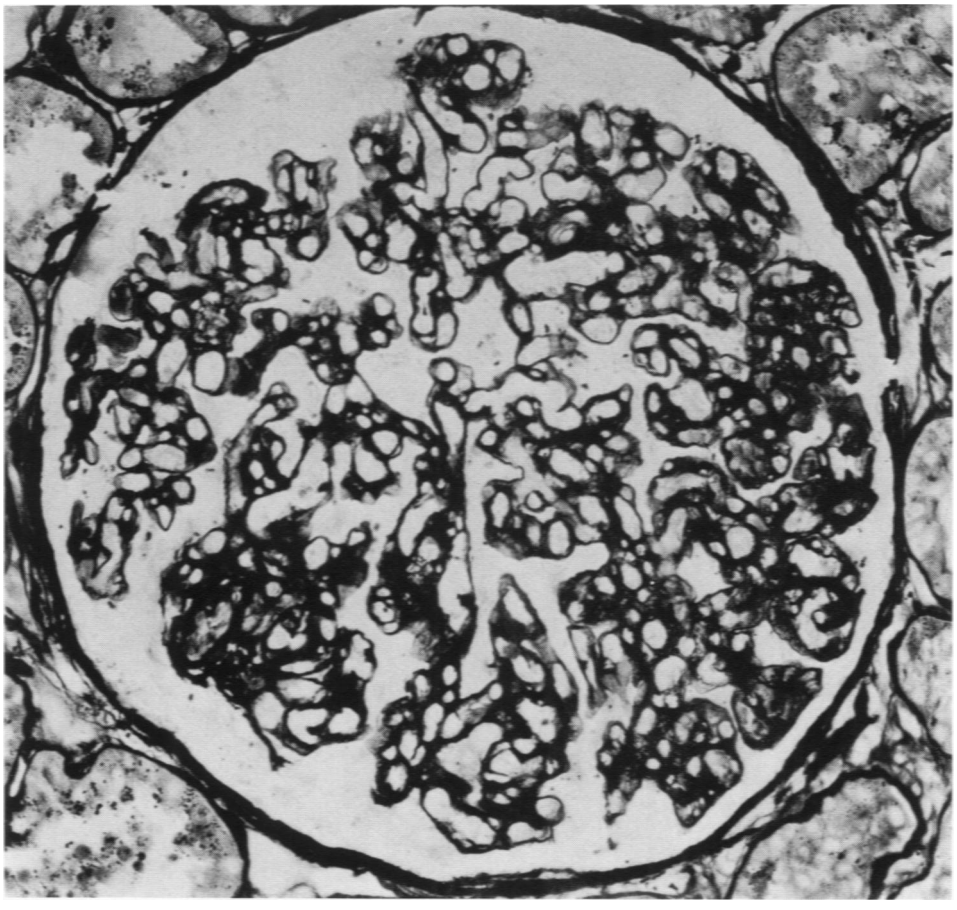

Fig 1—This greatly hypertrophied glomerulus from a patient with sickle cell anemia and the nephrotic syndrome (71-408) has many capillary loops with reduplicated GBMs. The mesangial matrix is increased in amount (Periodic acid methenamine silver, × 390).

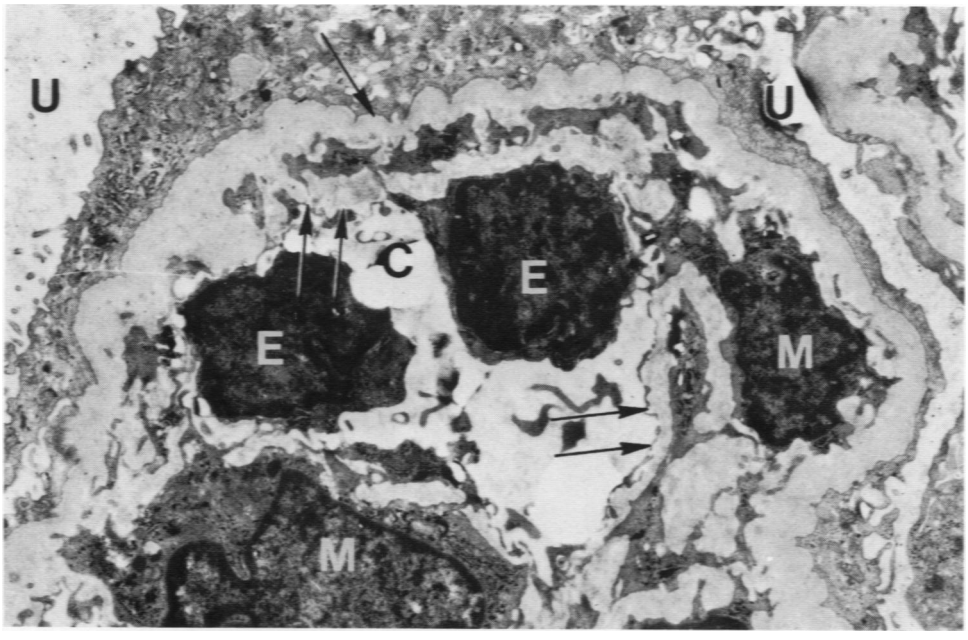

Fig 2—This electronmicrograph of a glomerular capillary loop from a patient with SSNS (70-5155) shows almost total reduplication of the GBM. On the right, a mesangial cell (*M*) is interposed between the original GBM (*single arrow*) and the new basement membrane (*double arrows*). *C*=capillary lumen, *U*=urinary space, *E*=endothelial cells (× 6000).

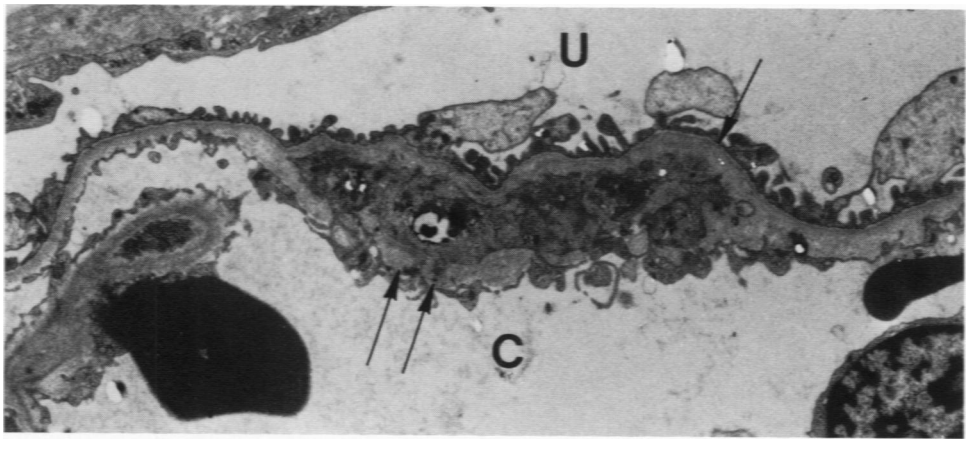

Fig 3—Peripheral capillary loop from patient with SSNS (72-6041) has only focal reduplication of the GBM. The new basement membrane (*double arrows*) is separated from the original GBM (*single arrow*) by mesangial cell cytoplasm. Neither dense deposits nor fibrin were seen in these regions (× 4500).